Adding a reporter gene to a conditional knockout model

A conditional knockout model with an integrated reporter makes your experiments easier and more certain. These lines function just like standard conditional knockouts, with the addition of a reporter that comes on after recombination has occurred. Add a reporter and carry out your experiments with certain knowledge of gene knockout in targeted cells.

Why add a reporter?

Knockout and validate with a single experiment.

Validating knockout in the tissue of interest is crucial whenever a Cre line is crossed to a conditional knockout line. This is especially important when one or both of the lines is new or being used in a new way. However, demonstrating that a Cre line and cKO line are interacting in the expected manner can be difficult. Depending on the gene that's the target of the knockout it may be challenging to demonstrate knockout has occurred, for example if good antibodies aren't available. Adding a reporter solves this - its expression depends on successful recombination in the targeted allele.

Accurately label cells where recombination has occurred.

Newly-made Cre lines are often crossed with generic reporter lines to demonstrate tissue-specific Cre expression. Generally the reporter line will express high levels of a protein such as β-galactosidase which is subsequently detected. This method can obscure different levels of Cre expression between tissues. Also, recombination that activates the generic reporter may not reflect how Cre will act on another gene. Different genomic regions have different succecptibility to recombination. Adding a reporter to a targeted allele ensures that cells are only labeled if there is recombination of that allele.

Adding a reporter to a conditional knockout model addresses both major issues with other validation strategies, and has an additional advantage: recombination is labeled by an easily detectable reporter. The reporter is within the target locus and can not activate unless there is recombination at that location. Thus validation of knockout is accomplished as part of the experiment. Finally, identifying reporter-expressing cells is usually very straightforward. Depending on the strategy for adding the reporter, bright fluorescence may be seen under a microscope, or the reporter labeled using an easily-obtained antibody.

Two Popular Strategies for Adding a Reporter to a Conditional Knockout Model:

Inverted reporter gene strategy

• Cre recombinase is most commonly used to conditionally delete sequences flanked by loxP sites. However, by using atypical sequences and orientation for the lox sites it is possible to invert the flanked sequence rather than delete it.

• Taking advantage of this, a reporter gene can be placed next to a targeted exon, with the reporter sequence oriented in the opposite direction to the target gene. Recombinase activity inverts the region and any exons that are inverted can no longer become part of the transcript. At the same time the reporter is placed in frame with the coding sequence, triggering its expression.

• The reporter sequence ends with a poly-A to help ensure early termination of the target gene's transcript. Through this disruption the gene is inactivated.

TruView Conditional Knockout™ *only from ingenious*

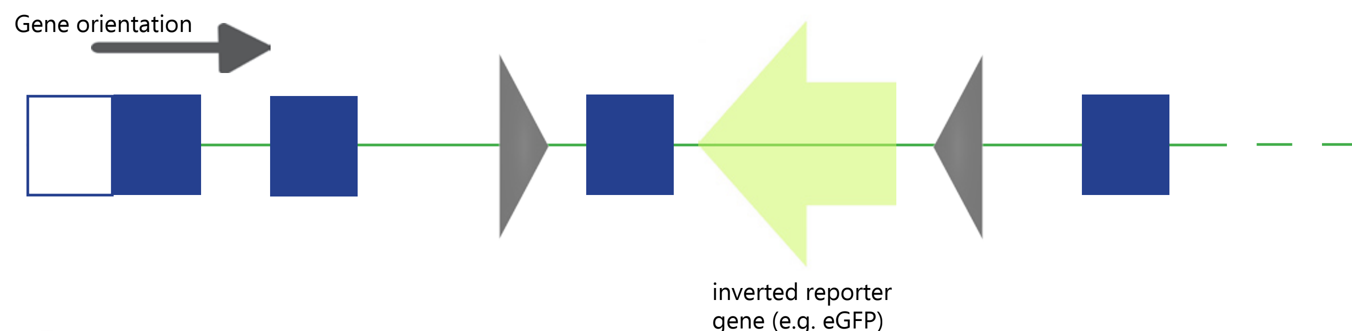
• ingenious' proprietary TruView strategy ensures that reporter expression is as bright as possible in all cells where recombination has occurred. This design splits the sequence of GFP into two parts which are brought together in the process of disrupting the targeted gene.

• A strong, exogenous CAG promoter drives GFP expression to create the strongest possible signal.

• This strategy makes it easier to flox larger regions - target an entire gene for deletion and be confident that knockout has occurred.

Inverted reporter strategy details

A general scheme of a gene targeted for conditional knockout with an inverted reporter:



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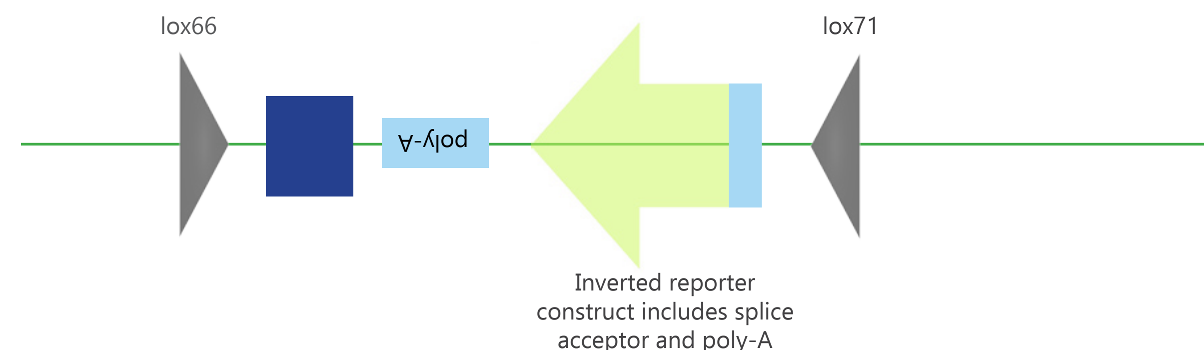
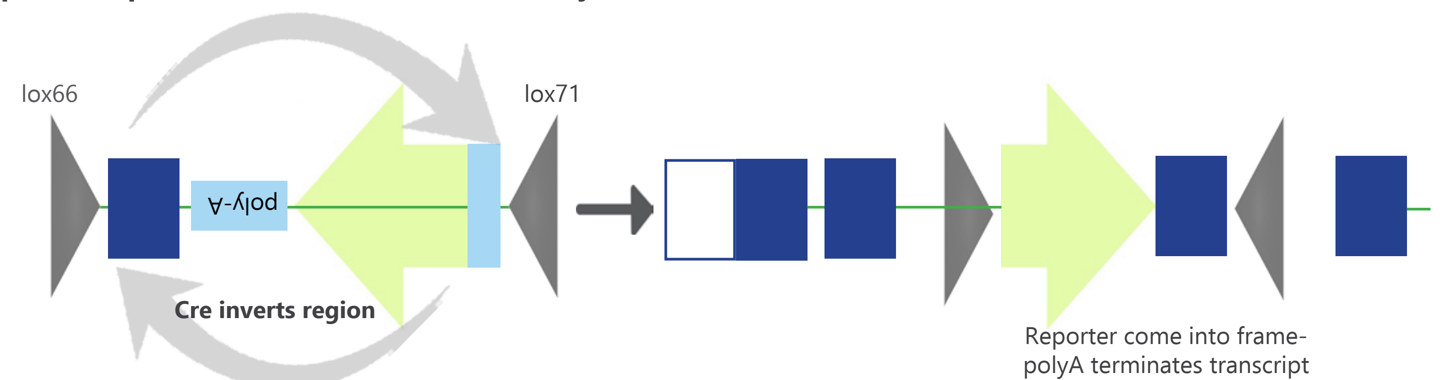
A more detailed schematic of the floxed region

(because reporter sequence is inverted compared with the orientation of the gene it is spliced over):

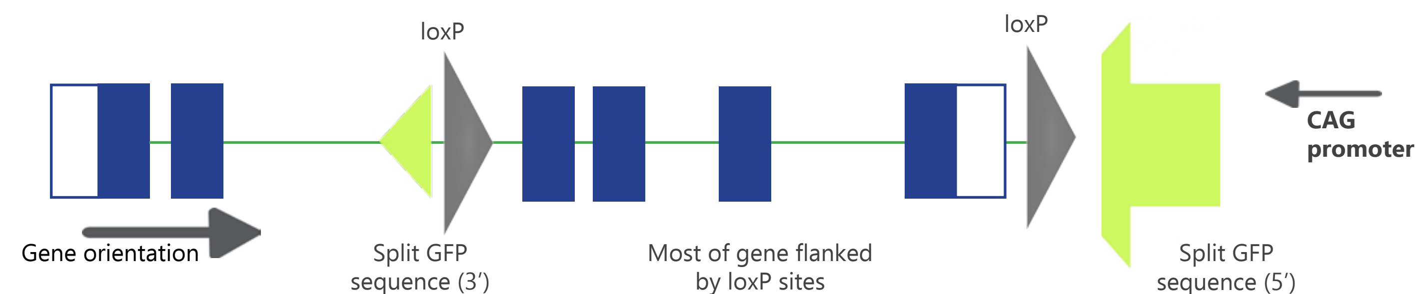


Cre takes target exon out of frame by inverting it, and brings reporter into frame. Gene is disrupted and reporter expression activated simultaneously:



TruView strategy details

A large portion of the target gene is flanked by loxP sites. A CAG promoter is placed downstream of the gene along with a partial GFP sequence:



After recombination most of the gene is deleted. The two partial GFP sequences are brought together and strong reporter expression is driven by the CAG promoter:

Additional strategies are available. Contact us today about a custom mouse, rat, or rabbit model.

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